mutation in a presenilin gene combined with a candidate drug.

The Pending Claims:

Prior to entry of the above amendments, Claims 1-12 are pending. Claims 1-9 are directed to a method for identifying candidate drugs for their potential efficacy for Alzheimer's disease with an electrophysiological screening method. Claims 10 – 12 are directed to mouse hippocampal cells combined with a candidate drug.

The Office Action:

The listing of references in the specification is not a proper Information Disclosure Statement under 37 CFR 1.98(b) and MPEP § 609.

Claims 1-12 are provisionally rejected under 35 U.S.C. § 101 as claiming the same invention of that of copending application 09/193,221.

Claims 1-12 are rejected under 35 U.S.C. § 112, first paragraph, as claiming subject matter which was not properly described in the specification.

Claims 1-9 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter.

Claims 6 and 8-9 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter.

Claims 10 and 11 are rejected under 35 U.S.C. § 102(b) as being anticipated by Borchelt *et al*.

Amendments:

Claim 1 has been amended in part by adding text to the phrase 'contacting mutant hippocampal cells, -- with a presenilin gene mutation -- having enhanced synaptic potentiation

upon stimulation'. This change more precisely specifies the types of cells used. The use of 'presenilin gene mutation' is supported by the specification, page 23, line 24, and claim 2 as filed. The word -- hippocampal -- was added to the phrase 'subjecting said mutant -- hippocampal – cells' to be more consistent with the rest of the text and is supported by page 3, line 22 of the specification. "Agent" was deleted and -- candidate drug -- added in its place since there was insufficient antecedent basis for "agent".

Claim 2 has been canceled.

Claims 3 and 4 have been amended to by changing "A" to -- The -- at the beginning of the claims. Claim 3 has been amended in part by deleting "said mutant cells are" and adding -- comprise said mutant hippocampal cells -- to the end of the claim. Support for this change is provided by Claim 3 as filed.

Claim 5 has been amended in part with the same changes to Claim 1. The word -- said -- was added before 'wild-type hippocampal cells' to establish an antecedent basis. The words -- hippocampal cells -- have been added after 'mutant' to be more consistent with the rest of the text of the claim, and is supported within the claim.

Claim 6 has been amended in part with the addition of the phrase -- for determining whether a mutation in hippocampal cells acts on a common pathway with a $GABA_A$ receptor antagonist, said method comprising -- in place of "according to Claim 5, including the additional steps of" since this claim is sufficiently distinct from claim 5 to warrant the change. This is supported by the last line of the claim. Changes to 'synaptic potentiation with time of "the" -- said -- mutant hippocampal cells' for consistency with the rest of the claim.

Claim 7 was amended by deleting the word "agent" and adding -- candidate drug -- in its place, since there was insufficient antecedent basis for "agent".

Claim 8 was amended with the same changes that were made to Claim 5.

Claim 9 has been amended in part with the same changes as in claim 5 and by adding the word -- said -- and deleting "the" for the sake of consistency within the claim. "Agent" was deleted and -- candidate drug -- added in its place since there was insufficient antecedent basis for "agent".

Claim 10 has been amended in part with the addition of the phrase -- that is not an antibody -- at the end of the claim. None of the examples of candidate drugs in the descriptions of the figures or in the specification or claims are antibodies, so this addition to the claim is supported by the text of the application. In Claims 10-12, "Slices of mouse hippocampal cells" now reads 'Slices of mouse hippocampal tissue -- containing cells –' since this phrase imparts more meaning to the claims (tissue, rather than cells, are what is being sliced). The latter change is supported in several locations in the specification, particularly page 6, line 21 and page 10, line 11.

Claim 12 was amended in part with the deletion of "the" and the addition of -- a -- before PS-1 Δ9 mutation.

Newly added Claim 13, , finds support in originally filed Claim 1, page 5 lines 12-14 and page 6, lines 16 – 19.

No new matter has been added by the amendments and the Examiner is respsectuflly requested to enter them.

Response to Rejections:

In the response that follows, the Examiner's specific objections and rejections are reiterated in small bold indented print, followed by Applicants' response, which is identified by normal print.

Information Disclosure Statement:

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(l) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

An information disclosure statement listing the references cited on page 3 is attached for review by the Examiner.

35 U.S.C. 101, Double Patenting

Claims 1-12 provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-12 of copending Application No. 09/193,221. This is a provisional double patenting rejection since the conflicting claims have not in fact been patented.

Applicants were notified on 9-6-00 that claims 1-12 in instant application are identical to those of the '221 application. However, no preliminary amendment has yet been received to correct the overlapping subject matter.

The rejection is avoided since the parent application is now abandoned in favor of the instant application, and the issue is thus moot. Accordingly, the Examiner is respectfully asked to withdraw the provisional rejection.

35 U.S.C. 112, first paragraph

Claims 1-12 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The claimed invention is drawn to methods for screening for drugs for the treatment of Alzheimer's Disease comprising "contacting mutant hippocampal cells having enhanced synaptic potentiation upon stimulation as compared to wild-type hippocampal cells with a candidate drug". One skilled in the art recognizes that it would require undue experimentation to practice the invention drawn to methods for screening for drugs given that claimed "mutant hippocampal cells" encompass hippocampal cells with any number of a myriad of mutations, e.g. mutations in a presenilin gene, as encompassed by the claims. One skilled in the art recognizes that modification of nucleotide sequence elements as claimed, e.g. mutations in a presenilin gene, produces nucleotide sequences that mediate unpredictable effects on gene function, for example unpredictable effects on regulation of expression of the genes operably linked to promoter region. For example, in view of the teachings of Smith et al (see PTO-892) and Darnell et al (see PTO-892) that modification of nucleotide sequences, for example by substitutions or other mutations including alterations of the reading frame, initiation codon, stop codon, and/or alignment of exons, produces nucleotide sequences that mediate unpredictable effects with regard to biological function of the mutated gene, e.g. cellular synaptic

function, one skilled in the art recognizes that the specification does not enable one skilled in the art to reliably and reproducibly predict the function, e.g. synaptic function, of "mutant hippocampal cells" in view that mutations mediate unpredictable effects on biological function. Further, the above teachings indicate that the ability of one skilled in the art to ascertain/assess the effects of "screening for drugs for the treatment of Alzheimer' s Disease" is unpredictable given that even a single gene mutation can alter the biological function of claimed hippocampal cells in an unpredictable manner. One skilled in the art would require undue experimentation to predictably ascertain the effects of drugs in methods to determine "activity of a candidate drug for the treatment of Alzheimer's Disease" as claimed.

Although Applicants to not agree with the Examiner's position, in order to further prosecution independent Claims 1, 5, 6, 8 and 9 have been added by adding the phrase -- with a presenilin gene mutation -- in reference to the mutant hippocampal cells. The claims as amended recite a specific class of cells upon which tetanic stimulation has been shown by Applicants to have enhanced synaptic stimulation as exemplified by cells with a PS-1 Δ9 mutation. Those skilled in the art would be able to make and use the claimed invention without undue experimentation.

The rejection of Claims 10-12 is traversed because the claims as filed recite slices of mouse hippocampal cells having a mutation in a presenilin gene; how to make and use these cells is described in the specification and therefor one skilled in the art would be able to make and use the claimed invention.

Accordingly, the Examiner is respectfully asked to withdraw the rejection.

Further, in view of the teachings of Parent et al (see PTO-892), one skilled in the art recognizes that the claimed invention, drawn to methods for screening for drugs for the treatment of Alzheimer's Disease comprising "contacting mutant hippocampal cells having enhanced synaptic potentiation upon stimulation as compared to wild-type hippocampal cells with a candidate drug", is unpredictable given that Parent et al teach results that are contradictory to the claimed invention (see below). In particular, Parent et al teach that, upon stimulation of mutant hippocampal cells (i.e. mutant hippocampal cells produced by a mutation in a presenilin gene) by stimulation with a tetanic stimulus (i.e. either theta-burst tetanic stimulation or high-frequency tetanic stimulation), no differences were observed, i.e. differences with regard to potentiation in response to tetanic stimulation, between mutant hippocampal cells (e.g. cells with presenilin- 1 mutation) as compared to wild-type hippocampal cells (see entire reference). Thus in view of the teachings of Parent et al, one skilled in the art recognizes that it would require undue experimentation to practice the claimed invention drawn to "mutant hippocampal cells having enhanced synaptic potentiation upon stimulation as compared to wild-type hippocampal cells", since Parent et al teaches that mutant hippocampal cells do not predictably or reliably have "enhanced synaptic potentiation upon stimulation" as compared to wild-type hippocampal cells.

Claims 1-9 are drawn to methods for screening for drugs comprising using "mutant hippocampal cells having enhanced synaptic potentiation upon stimulation as compared to wild-type hippocampal cells". In these claims as amended, cells that have presenilin gene mutations and enhanced synaptic potentiation after tetanic stimulation are used. This avoids the Examiner's rejections to which various mutations in hippocampal cells could mediate unpredictable or unreliable effects by requiring the use of cells with specific mutations that demonstrate the defined and desired effects. Mutant hippocampal cells that do not *reliably* have enhanced synaptic potentiation upon stimulation as compared to wild-type hippocampal cells are not within the scope of the claims.

We respectfully disagree with the Examiner's conclusion that the Parent *et al.* citation teaches that "upon stimulation of ... mutant hippocampal cells produced by a mutation in a presenilin gene by stimulation with a tetanic stimulus ... no differences were observed, i.e., differences with regard to potentiation in response to tetanic stimulation, between mutant hippocampal cells ... as compared to wild-type hippocampal cells". A careful reading of Parent *et al.* teaches the opposite, and contradicts the Examiner's contention. The Parent article does compare some basic parameters of synaptic transmission as well as long-term potentiation in neurons of non-transgenic controls and transgenic mice "expressing the wild-type PS1 gene or the PS1 variant (ref. P. 57, column 1, lines 8-12). Several basic parameters of synaptic transmission are measured, including "maximum fEPSP slope, maximum fEPSP amplitude, and maximum fiber volley amplitude" (ref. p. 58, column 1, lines 1-3). It should be noted that in the manner applied, these measurements do not induce long-term potentiation (LTP) since tetanic stimulation is not applied. Also analyzed are "LTP-inducing tetanic stimulus (either theta-burst or high-frequency)" waveforms following synaptic stimulation (ref. p. 58, column 2, lines 5-6). In the former measurements of non-LTP-inducing stimulus, "no differences were observed in these measures with these sets of animals" (ref. P. 58, column 1, lines 3-4). This is also taught by the specification of the instant application (Figure 1, description, page 3, line 30 through page 4, line 9). However, in measurements of LTP when theta burst was applied in the Parent article, "the amplitude of LTP in [mutant] animals was initially larger...and was more persistent than

that in [wild-type] or [non-transgenic] animals" After LTP-inducing high-frequency stimulation, "the difference in the magnitude of LTP between [mutant] and [wild-type] or [non-transgenic] mice was larger during the first 5 to 30 min" (ref. P. 58, column 1, lines 32-34). In their discussion of the aforementioned observations, the authors "report that transgenic mice expressing FAD-linked A246E human PS1 variants (MTg) [i.e., the transgenic mice bearing the human mutation] show a significant increase of fEPSP slope amplitude following tetanic stimulation when compared with NTg [non-transgenic mice] or WtTg [transgenic human wild type gene] controls".

We thus respectfully counter the Examiner's contention that there were "no .... differences with regard to potentiation in response to tetanic stimulation, between mutant hippocampal cells ... as compared to wild-type hippocampal cells" with the observations actually presented in the Parent article. The Parent article clearly demonstrates the differences observed between cells from the different sources when an LTP-inducing stimulus is applied, and in fact, points out these differences as the central focus of the article in the first sentence of the Discussion. Furthermore, since Parent *et al.* do in fact teach that mutant hippocampal cells have predictably and reliably enhanced synaptic potentiation upon ***tetanic*** stimulation as compared to wild-type hippocampal cells ("this ***increase*** becomes apparent within 5 min ***following*** the ***tetanus and becomes more prominent*** during the next 30 min."(ref. page 61, column 1, lines 12-14)), the assertion that undue experimentation would be required is inappropriate, since both the Parent article and the claims in this application teach the requirement for subjecting cells to tetanic stimulation. Applicants have taught that there are no differences between mutant and wild-type cells following non-LTP inducing stimuli (please see description for Figure 1, page 3, line 30 through page 4, line 9), but differences do exist between mutant and wild-type cells following tetanic stimulation (please see descriptions for Figures 2 through 5). Applicants have taught throughout the specification how to perform the claimed methods and how to obtain the cells. Accordingly, the Examiner is respectfully asked to withdraw the rejection.

Further, the specification provides insufficient guidance on how to successfully practice the invention as claimed because it is further unknown to one skilled in the art what metes and bounds are envisioned by the recitation "treatment", and because no *in vivo* models are known, or adequately described, for the "treatment of Alzheimer's disease" as claimed, by which the skilled artisan could extrapolate "how to use" the invention with

any reasonable expectation of success, for the reasons indicated above.

The applicants respectfully point out the following text from the MPEP:

MPEP 2107.02 I The applicant does not have to prove that a correlation exists between a particular activity and an asserted therapeutic use of a compound as a matter of statistical certainty, nor does he or she have to provide actual evidence of success in treating humans where such a utility is asserted. Instead, as the courts have repeatedly held, all that is all that is required is a reasonable correlation between the activity and the asserted use.

We respectfully argue that the language of the MPEP is clear and pertinent. That is, all that is required is a that reasonable correlation exists between an activity, in this case, electrophysiology as practiced in the claimed invention, and the asserted use, which is a screening method for candidate drugs, rather than a treatment.

We also note that the metes and bounds of treatment are irrelevant. Claims 1-5 and 7-9 disclose a method for screening drugs, not for treating humans. This is evidenced by the wording of the phrase used in the independent claims 1, 5, 8, and 9: "A method for screening drugs....*is indicative of a candidate drug* for the treatment of Alzheimer's disease. The use of the word "candidate" does not infer anything about treatment; that a particular drug is efficacious *in vivo*, that it is safe, that it will be profitable, or that it will ever be used. Rather, it implies that a compound has been identified with a screening process as one that *might* demonstrate efficacy and likely merits further testing. "The scope of ennoblement must only bear a 'reasonable correlation' to the scope of the claims" (MPEP 2164.08), and, in this case, the scope of the claims is a screening method, not a treatment. The reasons why this particular screening method is related to identifying drugs is disclosed in the specification, in that the measurement of a reduction of aberrant signaling may be used to indicate the presence of a candidate drug (page 3, lines 22-26).

Claim 6 discloses a method for determining whether a mutation in hippocampal cells acts on a common pathway with a $GABA_A$ receptor antagonist, again, not a method for treating humans. The method disclosed in the claim is "subjecting said mutant hippocampal cells and said wild type cells to tetanic stimulation". There is no indication in the claim that said

tetanic stimulation is conducted *in vivo*.

> **... because no *in vivo* models are known, or adequately described, for the "treatment of Alzheimer's disease" as claimed, by which the skilled artisan could extrapolate "how to use" the invention with any reasonable expectation of success, for the reasons indicated above. Additionally, it is well accepted in the art that differences exist between *in vitro* protocols and results, e.g. *in vitro* results from studies using hippocampal slices as disclosed, versus *in vivo* protocols and results, e.g. *in vivo* studies that employ drug administration, especially as it relates to undefined parameters that do not distinguish when "treatment" is effective, or that require passage across the blood brain barrier which is impermeable to protein molecules/other molecules, or that involve undefined parameters that do not distinguish "treatment" of "Alzheimer' s Disease", for example, from any different disease state. The instant specification provides insufficient guidance on how these parameters are to be determined, how a similar method was practiced in the art with a different agent or to provide even a single working *in vivo* example of the claimed methods.**

We respectfully argue that these contentions have no bearing on the instant claims for five distinct and individually sufficient reasons.

First, this instant invention is a method for screening drugs *in vitro*, not for determining *in vivo* protocols or "passage across the blood brain barrier", the latter being totally irrelevant.

Second, it is well known to ordinary artisans that screening methods are discovery methods. *In vivo* models and clinical trials provide drug *efficacy*, *validation* and *safety* testing, not *discovery*. This is particularly well known to those skilled in the art, none of whom can afford to discover drug candidates using live subjects.

Third, numerous murine models of Alzheimer's disease with presenilin mutations exist. These include but are not limited to the models of Lamb *et al.*, (Nature Neuroscience, 1999, vol. 2, (8), p. 683 – 771), Janus *et al.*, Neurobiol. Aging **21**:(4) 541-549) and Bournemann *et al.*, (Ann. N.Y. Acad. Sci., 2000, vol 908, p. 260-266) (please see Appendix).

Fourth, the MPEP (2107.02 IV) clearly points out:

> MPEP 2107.02 III Office personnel should be careful not to find evidence unpersuasive simply because no animal model for the human disease condition has been established prior to the filing of the application. ... The mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it.
>
> MPEP 2107.02 IV Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials. There is no decisional law that requires an applicant to provide data from human clinical trials to establish utility for an invention related to treatment of human disorders...even with respect to situations where

no art-recognized animal models existed for the human disease encompassed by the claims.

Thus, a lack of an *in vivo* model (which is not the case here) does not imply that the evidence for utility is unpersuasive. A rejection based on the absence of clinical data is imposing "on applicants the unnecessary burden of providing evidence from human clinical trials". Furthermore, treatment studies in humans are not relevant to the drug screening process.

Finally, drugs are often investigated and marketed for diseases with no adequate *in vivo* models (e.g. androgen-independent and metastatic human prostate disease), for which *in vitro* models currently provide the methods for drug discovery.

Additionally, it is not known at what point during any given disease state of Alzheimer's disease when "treatment" is recommended, or how one skilled in the art knows when, or if, they have successfully practiced the instant invention; thereby, requiring undue experimentation to discover how to successfully practice Applicants' invention. Further, it is unknown, nor disclosed, what specific aspects/symptoms of claimed Alzheimer' s disease are envisioned to be "treated" or what constitutes a therapeutically effective amount of claimed "candidate drug", or how to assay such *in vivo*. In other words, one skilled in the art would not reasonably be able to successfully make and use the invention, as claimed, without undue experimentation to determine such.

We submit that individuals skilled in the art of drug screening know they have successfully practiced the instant invention when they have eliminated some candidate drugs and identified others of greater interest, said activity being the motivation behind drug screening. Said individuals are more likely to succeed with a few "hot leads" as opposed to a large number of drug candidates of varying potential, and be rewarded for their economy. Any method that reduces the pool of candidate drugs before clinical trials has merit as a method of screening drugs.

Drug screens have nothing to do with determining therapeutic indexes. If a candidate drug has any biological activity of interest, it may move to the next phase of testing, said move being more likely if the activity is above a threshold level as measured in the drug screen. Therapeutic doses are determined after much effort is made in purification, chemical modification (in many cases), matrix evaluation and toxicity testing. Drug screens make take place if there is yet no means to determine a "therapeutically effective amount." If no drug candidate is found, as if often the case, there is no need to acquire, for example, toxicity data. Similarly, determining when treatment is recommended is irrelevant to the disclosed invention

because drugs, and not drug candidates, are used as treatments.

Accordingly, the Examiner is respectfully asked to withdraw the rejections.

35 U.S.C. 112, second paragraph

Claims 1-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Said claims recite the limitation "said agent". There is insufficient antecedent basis for this limitation in the claims.

This rejection has been avoided by amendment of Claims 1, 5, 7, 8 and 9 by changing "agent" to -- candidate drug --. This avoids the Examiner's rejection, in which the limitation "said agent" in the original wording lacked antecedent basis. Accordingly, the Examiner is respectfully asked to withdraw the rejection.

Claims 6 and 8-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The term "significant change" in said claims is a relative term which renders the claims indefinite. The specification does not provide a standard for ascertaining the requisite degree of change required to exactly constitute "significant change", and one skilled in the art would not be reasonably apprised of the scope of the invention.

The applicants respectfully point out the following text from the MPEP:

> MPEP 2107.01 VII There is no predetermined amount or character of evidence that must be provided by the applicant to support an asserted utility, therapeutic or otherwise. ..., Furthermore, the applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." *In re Irons*, 340 F.2d 974,978, 144 USPQ 351, 354 (CCPA 1965). Nor must an applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty. *Nelson vs. Bowler*, 626 F.2d 853, 856-57, 206 USPQ 881, 883-84 (CCPA 1980) (reversing the Board and rejecting Bowler's arguments that the evidence of utility was statistically insignificant. The court pointed out that a rigorous correlation is not necessary when the test is reasonably predictive of the response). ... Instead evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely true than not true.

Claims in U.S. patents generally do not include levels of statistical significance in spite of the myriad use of phrases such as "greater than", "less than", "significant change" and the like. We argue that, taken as a whole (including the statistical significance provided by the

descriptions of the relevant figures and the associated data), the specification provides support for the claims. The Examiner states "the specification does not provide a standard for ascertaining the requisite degree of change required to exactly constitute 'significant change' ". Applicants submit that the claims do not provide evidence that establish the asserted utility as a matter of statistical certainty, or operate from a standard that ascertains "a requisite degree of change", and there is no requirement to do so. On the contrary, applicants may avoid the use of levels of significance: "Nor must an applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty" (MPEP 2107.01 VII). Accordingly, the Examiner is respectfully asked to withdraw the rejection.

35 U.S.C. 102(b) Rejection

Claims 10 and 11 are rejected under 35 U.S.C. 102(b) as being anticipated by Borchelt et al (October 1997 reference; see PTO-892). Instant claim 10 is drawn to slices of mouse hippocampal cells having a mutation in a presenilin gene "combined with a candidate drug". Since Borchelt et al (see entire reference) disclose slices of mouse hippocampal cells having a mutation in a presenilin gene "combined with a candidate drug", e.g. see Figure 5 of Borchelt et al reference for disclosure of slices of mouse hippocampal cells having a mutation in a presenilin gene combined with antibodies (i.e. antibodies meet limitation of any candidate drug) specific to the C-terminus of beta-amyloid 1-40 and beta-amyloid 1-42. Thus in view of the disclosure of Borchelt et al, all limitations of claim 10 are met by the prior art. Claim 11 is a product by process claim. There is no evidence of record that the process of tetanic stimulation materially affects the product slices of mouse hippocampal cells from the product as claimed in claim 10, see in particular evidentiary support of Parent et al wherein no predictable or reliable synaptic potentiation was achieved. Thus, it appears that the products are the same and claim 11 is anticipated by Borchelt et al., as set forth above.

The rejection of Claims 10 and 11 has been avoided by amendment of Claim 10 by combining the slices of mouse hippocampal cells with a candidate drug -- that is not an antibody.-- . Borchelt *et al.* therefore does not anticipate the newly worded claims. Furthermore, the "candidate drug" disclosed by Borchelt *et al.* is an antibody for immunostaining, a process conducted *in vitro*. Reagents used *in vitro* are not drugs, and are not recognized as such by the U.S. Food and Drug Administration. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

Parent *et al.* is not available as a reference. The instant application has a priority date of November 16, 1998, the parent application 09/193,221 having been filed on that date. The Parent *et al.* citation was published in February of 1999, which is after the priority date.

CONCLUSION

In view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 328-4400.

Respectfully submitted,

Dated: February 12, 2001

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Appendix

1. Bornemann, K.D., and M. Staufenbiel. 2000. Transgenic mouse models of Alzheimer's disease. *Ann. N. Y. Acad. Sci.* **908:** 260-266.
Abstract:
Alzheimer's disease (AD) pathology is characterized by A beta peptide-containing plaques, neurofibrillary tangles consisting of hyperphosphorylated tau, extensive neuritic degeneration, and distinct neuron loss. We generated several transgenic mouse lines expressing the human amyloid precursor protein (APP751) containing the AD-linked KM670/671NL double mutation (Swedish mutation) under the control of a neuron-specific Thy-1 promoter fragment. In the best APP-expressing line (APP23), compact A beta deposits can be detected at 6 months of age. These plaques dramatically increase with age, are mostly Congo Red positive, and accumulate typical plaque-associated proteins such as heparansulfate proteoglycan and apolipoprotein E. Activated astrocytes and microglia indicative of inflammatory processes reminiscent of AD accumulate around the deposits. Furthermore, plaques are surrounded by enlarged dystrophic neurites as visualized by neurofilament or Holmes-Luxol staining. Strong staining for acetylcholinesterase activity is found throughout the plaques and is accompanied by local distortion of the cholinergic fiber network. All congophilic plaques contain hyperphosphorylated tau reminiscent of early tau pathology. Modern stereologic methods demonstrate a significant loss of neurons in the hippocampal CA1 region, correlating with an increasing A beta plaque load. Interestingly, APP23 mice develop cerebral amyloid angiopathy in addition to amyloid plaques even though the APP transgene is only expressed in neurons. Crossbreeding of APP23 mice with transgenic mice carrying AD-linked **presenilin** mutations but not wild-type presenilin resulted in enhanced formation of pathology. In conclusion, our APP transgenic mice present many pathologic features, similar to those observed in AD and therefore offer excellent tools for studying the contribution of A beta to AD pathogenesis.

2. Janus, C., S. D'Amelio, O. Amitay, M. A. Chisti, R. Strome, P. Fraser, G. A. Carlson, J. C. Roder, P. St. George-Hyslop, and D. Westaway. 2000. Neurobiol. Aging. **21**:(4) 541-549.
Abstract:
Dominant mutations in the Presenilin 1 gene are linked to an aggressive, early-onset form of familial Alzheimer's Disease (FAD). Spatial memory of transgenic (Tg) mice expressing either mutant (lines Tg(M146L)1, Tg(M146L)76, Tg(L286V)198) or wild type (line Tg(PS1wt)195) human PS1 transgenes was investigated in the Morris water maze (WM) test at 6 and 9 months of age. The results showed that the mutated Tg mice had increased swim speed when compared to non-Tg littermates or Tg PS1 wild type mice. The swim speed difference did not, however, significantly affect the spatial learning in the WM

test and all groups showed comparable search paths during training and similar spatial bias during probe trials. When re-tested at 9 months, all mice showed significantly improved learning acquisition of spatial information. The lack of progressive spatial learning impairment in mice expressing the mutated human PS1 transgene in the WM does not preclude impairments in other cognitive tasks but suggests that full phenotypic expression of mutant PS1 alleles may require co-expression of human versions of other AD-associated genes.

3. Lamb, B.T., K. A. Bardel, L. S. Kulnane, J. J. Anderson, G. Holtz, S. L. Wagner, S. S. Sisodia and E. J. Hoeger. Amyloid production and deposition in mutant *amyloid precursor protein* and ***presenilin-1*** yeast artificial chromosome transgenic mice. 1999. *Nature NeuroSci.* **2**:(8):695 – 697.

Scientific Correspondence (no abstract)